

## **DETECTION OF DENGUE VIRAL RNA IN MOSQUITOES (*Aedes* SP.) BY NUCLEIC ACID SEQUENCE-BASED AMPLIFICATION (NASBA) AND REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION (RT-PCR)**

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Virologic surveillance for dengue viruses has been used as an early warning system to predict outbreaks. In this study, the NASBA technique was used to detect serotypes specific of dengue viruses in artificially-infected and in field-caught female adult *Aedes* mosquitoes, which were compared with the RT-PCR technique. In laboratory experiments, NASBA could detect dengue virus serotypes 2 and 4 below 1 PFU/ml, which was more sensitive than RT-PCR, but this technique was as sensitive as RT-PCR when detecting dengue virus serotypes 1 and 3. Most dengue viruses were found at the thorax of mosquitoes in 0, 7, and 14 days after inoculation with dengue virus serotype 2. In the field, female adult *Aedes* mosquitoes were collected in the rainy season (June-August, 2002) and the dry season (April, 2003) in Nong Khai Province. These mosquitoes were caught from selected dengue epidemic areas and assayed by NASBA, compared with RT-PCR. From 630 mosquito samples, viral infection rates were 1.61% and 1.13% for NASBA and RT-PCR detection, respectively. The results showed the mosquitoes were infected only with dengue virus serotypes 1 and 2. The sensitivity and specificity of NASBA when compared with PCR were 100% and 99.52%, respectively. The sensitivities of NASBA for two dengue virus serotypes were 100%, whereas the specificities were 99.68% and 99.84% for serotypes 1 and 2, respectively. For serotyping, NASBA showed similar sensitivity and specificity RT-PCR (= 0.70). These results indicate that the NASBA assay is another tool for the surveillance of infected mosquitoes that is useful for decreasing the dengue problem.

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## **EVALUATION OF A SINGLE-DILUTION PLAQUE REDUCTION NEUTRALIZATION TEST (PRNT) AS A DIAGNOSTIC TOOL FOR DETECTING INTERCURRENT DENGUE VIRUS (DENV) INFECTIONS**

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Studies of DENV transmission require diagnostic tools capable of detecting symptomatic and asymptomatic infections. The hemagglutination-inhibition (HAI) test is valuable for the diagnosis of DENV infections, but possesses low specificity. Since HAI antibody may wane, the HAI test as a DENV surveillance tool may require frequent cohort serum sampling. The PRNT is considered the gold standard bioassay for measuring DENV specific neutralizing antibody. The PRNT is labor-intensive and requires affinity maturation to provide specificity. Previously reported studies have relied on a single-dilution neutralization test (SDNT) to detect intercurrent